

SHORT COMMUNICATION

ELISA Based Anthrax Antibody Titer in Cattle Induced by Locally Prepared Anthrax Vaccine Originated from Sterne F-24 Strain in Bangladesh

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ABSTRACT

Vaccination is usually practiced to prevent and control anthrax in Bangladesh. For this purpose, vaccine prepared from Sterne F-24 strain of *Bacillus anthracis* by Livestock Research Institute (LRI), Mohakhali, Dhaka has long been used in this country. However, in some cases anthrax occurred in vaccinated animals in Bangladesh. A total of 100 cattle at Lal Teer Livestock Research and Development Farm, Lal Teer Livestock Limited, Bangladesh, aging between 3-6 years and weighing between 250-400 kg were randomly selected for vaccination purpose. Blood samples (n=100) were collected before the vaccination for collecting pre-vaccination serum, and the animals were vaccinated (at 1 mL/animal; 1×10^7 spores/mL) with the anthrax vaccine produced by LRI. All blood samples from the vaccinated animals were collected on day 7, 28, 60, 90, 120, 150, 180, 240, 270, 300, 330, and 360 of post-vaccination, and serum samples were prepared. The antibody levels in the serum samples against anthrax were monitored using an Enzyme-Linked Immunosorbent Assay (ELISA). Over the course of 12 months, the antibody titers were found at the level higher than the reference value. Though there were reports on anthrax suspected cases in this farm, no such cases were reported during the study period. Thus, the vaccine appears to induce adequate antibody response against anthrax in Bangladesh.

Keywords: Cattle, ELISA, Antibody titer, Vaccine, Sterne F-24 strain, Bangladesh

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Introduction

Anthrax (popularly known as “*Torka*” in Bangladesh) is an acute infectious disease caused by *Bacillus anthracis* (Weiss et al., 2007; OIE, 2008). Although the disease is found in almost all countries, it is most prevalent in tropical and sub-tropical countries (Biswas et al., 2011). In recent years, the disease has been reported in Sweden (Lewerin et al., 2010), Italy (Fasanella et al., 2010), USA (Mongoh et al., 2008), Australia (Durrheim et al., 2009), and some other countries in Europe. *B. anthracis* forms spore in unfavorable conditions, which is resistant to heat and chemical disinfectants, and this may persist in soil as viable for several decades (Hirsh and Zee, 1999; Dragon et al., 2001; OIE, 2004).

Primarily, herbivores like cattle, sheep, goat, horse and pig are affected with anthrax (Ahsan et al., 2013) and the disease is usually fatal in ruminants (Islam et al., 2013). In humans, anthrax is almost invariably contracted directly or indirectly from animals (WHO, 2008). In Bangladesh, the disease was sporadically reported in animals and human until 2009 (Ahmed et al., 2010). In recent years, the disease occurred repeatedly; the outbreaks indicated that the disease is no longer sporadic, rather enzootic in Bangladesh (Ahmed et al., 2010; Fasanella et al., 2013; Ahsan et al., 2013). In different areas of Bangladesh, 14 outbreaks occurred during the period of August 2009 to October 2010, affecting 140 animals and subsequently to 273 humans. This created a panic to the people. As a result in 2010, Bangladesh Government announced a ‘Red Alert’ throughout the country. Consequently, beef price and foreign exchange were decreased by 126% and 17% respectively, and about 35% small scale butchers were driven out of business in 2010 (Uddin, 2011). Along with social and environmental factor scarcity and limited vaccination was mentioned as one of the contributing factors of repeated outbreaks of anthrax in Bangladesh (Chakraborty et al., 2012; Islam et al.,

2013; Hassan et al., 2015).

Vaccination is usually practiced in Bangladesh to prevent anthrax in animals. For this vaccination, Sterne F-24 strain of *B. anthracis* originated from Australia is used in Bangladesh (Roy et al., 2013). A single dose of vaccine is given to each animal that protects the animal for one year from anthrax. The protective effect of a single dose of strain 34F2 vaccine is said to last about one year (Sterne, 1939). However, successful development of protective immunity against anthrax in animals requires effective vaccine and proper vaccination technique. Also, a single dose of Sterne vaccine may not be sufficient to ensure protective immunity in the animal to last for a year, and more than one initial dose of the Sterne vaccine may be necessary (Turnbull et al., 2004; WHO, 2008; Mongho et al., 2008). Very few studies focusing on immunological characterization have been conducted with cattle and goats, and the previous studies covered a limited period of time (Roy et al., 2013; Dipti et al., 2013; Nagarajan et al., 2015). Moreover, the previous studies have been conducted with a limited number of animals in experimental condition. Recently, we reported the causes of repeated anthrax outbreak in Bangladesh as well as the present status of knowledge, attitude, and behavior of people towards anthrax (Ahsan et al., 2013; Hassan et al., 2015). Here, we determined the titer of anthrax antibody level in cattle under commercial farm condition throughout the year using Enzyme Linked Immunosorbent Assay (ELISA).

Materials and Methods

The study was performed in a commercial animal facility at Lal Teer Livestock Research and Development Farm, Lal Teer Livestock Limited, Bangladesh, located at Bongao, Uthura, Valuka, Mymensingh, during the

period from April 2013 to April 2014. The anthrax vaccine was collected from the Livestock Research Institute (LRI), Mohakhali, Dhaka, Bangladesh. The master seed of this vaccine contained living spore of the non-capsulated attenuated Sterne F-24 strain of *B. anthracis* obtained originally from Australia. A total of 100 healthy and unvaccinated cattle at the farm were randomly selected for this study. The cattle received anthrax vaccine in previous years as a routine work. However, the cattle did not receive any anthrax vaccine within past one year when the study started. The cattle were aged between 3-6 years weighing between 250-400 kg.

For the determination of antibody level against anthrax, blood samples (10 mL each; n=100) were collected before vaccination of the animals. Then all the cattle were vaccinated following the schedule mentioned by LRI. Briefly, 1 mL vaccine containing 1×10^7 spores (approximately) was injected per animal through subcutaneous route at neck region. Repeat sets of blood samples (n=100) were collected from the vaccinated animals on day 7, 28, 60, 90, 120, 150, 180, 240, 270, 300, 330, and 360 of post-vaccination. All blood samples were carried in ice box to the Laboratory at the Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202. Serum samples were prepared from the blood samples according to the method described by Dipti *et al.* (2013). The antibody level in the serum samples were determined by ELISA (AniGenB. *anthracis*Ab ELISA kit, BIONOTE Inc., Republic of Korea).

Results and Discussion

The cattle vaccinated with local anthrax vaccine of Bangladesh were found to elicit sufficient immune response and production of antibody level above the reference value over a period of 12 months (Table 1; Fig. 1). According to the ELISA kit, samples to positive ratio (S/P ratio) ≥ 0.5 was indicative of vaccination or previous exposure to anthrax. The pre-vaccination S/P ratio obtained in this study was 0.16 ± 0.05 , indicated that the cattle had low level of antibody against anthrax, which could be produced during the previous year vaccination cycle. Vaccination triggered antibody production and was reached above the reference value at day 7 of post-vaccination (Table 1). The highest S/P ratio was recorded as 2.15 ± 0.17 at day 28 of post-vaccination (Fig. 1). The antibody levels over the year were found fluctuating giving a minimum S/P ratio of 1.07 ± 0.14 on day 180 of post-vaccination (Table 1). Though there were some reports of anthrax suspected cases in the study farm (personal communication), there was no reports of such cases during the study period, indicating that the vaccinated animals developed protective immunity against anthrax. However, due to the lack of experimental facilities we could not perform challenge experiment. Thus, it was not possible to determine the minimum protective antibody levels against anthrax infection induced by the local anthrax vaccine. Vaccination of animal with local anthrax vaccine followed by challenge exposure with virulent *B. anthracis* strains isolated from various host species would reveal the minimum protective antibody levels induced by the vaccine. Experimental evidence shows that antibody induced by Sterne strain could protect guinea pig against pathogenic *B. anthracis* isolated from different animals (Little and Knudson, 1986). In addition to the vaccine type, health status of animal is crucial to develop proper immune response (IOWA Beef Center website).

Vaccination response as obtained in this study differed from the finding of Dipti *et al.* (2013) and Roy *et al.* (2013) although they used same vaccine that we used in our study, who could detect early immune response in cattle at day 30 of immunization; whereas, in the present study we revealed early response at day 7 of post-vaccination. In addition, Dipti *et al.* (2013) described a steady increase in antibody level upto 90 days. In contrast, we found the antibody level was fluctuating over the study period (Fig. 1). The fluctuating nature of antibody in the body might be due to being the vaccine as live spore vaccine, and health status of the responders. Live vaccines after introduction into the body goes under multiplication, like BCG vaccine goes under multiplication in the injection site and in the local draining lymph induces profound inflammatory and immune responses by activating T and B lymphocyte (WHO website). Anything that alters the replication of the organisms in the body can cause altered immune response induced by the vaccine as well as render the vaccine become ineffective (CDC website). However, our findings strengthen the findings of Dipti *et al.* (2013) and Roy *et al.* (2013) who conducted the research works up to 90 days. In this study we report the results for an extended period of time upto one year.

Table 1: S/P ratios of ELISA tests of serum samples collected from the cattle vaccinated with LRI produced Anthrax spore vaccine. Samples to positive ratio (S/P ratio) ≥ 0.5 was indicative of vaccination or previous exposure to anthrax.

Serum collected at days of vaccination	S/P ratio (Mean \pm SE)
Day 0 (Pre-vaccination)	0.16 \pm 0.05
Day 7	1.31 \pm 0.17
Day 28	2.15 \pm 0.17
Day 60	1.34 \pm 0.16
Day 90	1.47 \pm 0.18
Day 120	1.08 \pm 0.18
Day 150	1.25 \pm 0.16
Day 180	1.07 \pm 0.14
Day 240	1.43 \pm 0.19
Day 270	1.53 \pm 0.12
Day 300	1.44 \pm 0.20
Day 330	1.80 \pm 0.18
Day 360	1.84 \pm 0.48

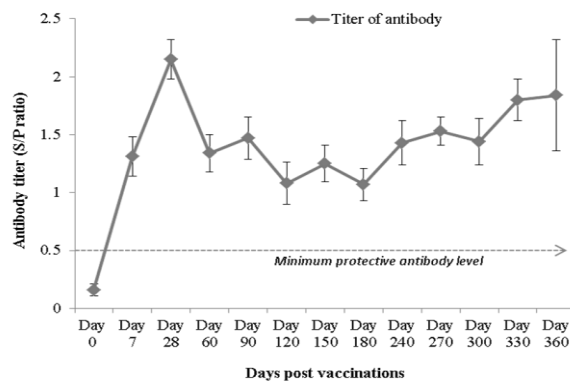


Figure 1. Antibody level of the vaccinated animals against anthrax. As per the instruction of the ELISA kit used in this study, 0.5 was considered as the minimum protective level (green arrow) against anthrax.

Conclusion

The anthrax vaccine manufactured by LRI, Mohakhali, Dhaka, Bangladesh could protect the vaccinated cattle from anthrax up to one year. Therefore, the anthrax vaccine produced by this institute may be effectively used to prevent anthrax outbreaks in animal population of Bangladesh. However, further study of the immunogenicity of the vaccine along with challenge exposure is recommended to establish whether, the antibody induced by the vaccine is protective or not.

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References

- Ahmed, N., Sultana, Y., Fatema, DSM., Ara, K., Begum, N., Mostanzid, SM., and Jubayer, S. (2010). Anthrax: An emerging zoonotic disease in Bangladesh. *Bangl J Med Microbiol*, 4: 46-50.
- Ahsan, MM., Khan, MFR., Rahman, B., Hassan, J., Chowdhury, SMZH., Parvej, MS., Jahan, H., and Nazir, KHMNH. (2013). Investigation into *Bacillus anthracis* spore in soil and analysis of environmental parameters related to repeated anthrax outbreak in Sirajganj, Bangladesh. *Thai J Vet Med*, 43: 449-454.
- Biswas, PK., Islam, MJ., Shil, SK., Chakraborty, RK., Ahmed, SSU., and Christensen, JP. (2011). Risk factors associated with anthrax in cattle on smallholdings. *Epidemiol Infect*, 140: 1888-1895.

- Chakraborty, A., Khan, SU., Hasnat, MA., Parveen, S., Islam, MS., Mikolon, A., Chakraborty, RK., Ahmed, BN., Ara, K., Haider, N., Zaki, SR., Hoffmaster, AR., Rahman, M., Luby, SP., and Hossain, MJ. (2012). Anthrax outbreaks in Bangladesh, 2009-2010. *Am J Trop Med Hyg*, 86: 703-710.
- Dipti, M., Rashid, MM., Ferdoush, MJ., Roy, P., Khan, MAHNA., and Hossain, MM. (2013). Morphological and immunological characterization of anthrax vaccine in cattle. *Bangl J Vet Med*, 11: 43-49.
- Dragon, DC., Rennie, RP., and Elkin, BT. (2001). Detection of anthrax spores in endemic regions of northern Canada. *J Appl Microbiol*, 91: 435-441.
- Durrheim, DN., Freeman, P., Roth, I., and Hornitzky, M. (2009). Epidemiologic questions from anthrax outbreak, hunter valley, Australia. *Emerg Infect Dis*, 15: 840-842.
- Fasanella, A., Garofolo, G., Galante, D., Quaranta, V., Palazzo, L., Lista, F., Adone, R., and Jones, MH. (2010). Severe anthrax outbreaks in Italy in 2004: considerations on factors involved in the spread of infection. *New Microbiol*, 33: 83-86.
- Fasanella, A., Garofolo, G., Hossain, MJ., Shamsuddin, M., Blackburn, JK., and Hugh-Jones, M. (2013). Bangladesh anthrax outbreaks are probably caused by contaminated livestock feed. *Epidemiol Infect*, 141: 1021-1028.
- Hassan, J., Ahsan, MM., Rahman, MB., Chowdhury, SMZH., Parvej, MS., and Nazir, KHMNH. (2015). Factor associated with repeated outbreak of anthrax in Bangladesh: qualitative and quantitative study. *J Adv Vet Anim Res* (Online First). doi:10.5455/javar.2015.b72
- Hirsh, DC., and Zee, YC. (1999). The Genus *Bacillus*. In: *Veterinary Microbiology*. Ernst L, Berstein B, Hirsh DC (Edn.), Blackwell Science Inc., USA; pp 246-249.
- IOWA Beef Center website. Beef cattle handbook by Richard C. Bull. http://www.iowabeefcenter.org/Beef%20Cattle%20Handbook/Trace_Minerals_Immunology.pdf. Accessed on February 1, 2015.
- Islam, MS., Hossain, MJ., Mikolon, A., Parveen, S., Khan, MSU., Haider, N., Chakraborty, A., Titu, AMN., Rahman, MW., Sazzad, HMS., Rahman, M., Gurley, ES., and Luby, SP. (2013). Risk practices for animal and human anthrax in Bangladesh: an exploratory study. *Infect Ecol Epidemiol*, 3: 21356.
- Lewerin, SS., Elvander, M., Westermark, T., Hartzell, LN., Norström, AK., Ehlers, S., Knutsson, R., Englund, S., Andersson, AC., Granberg, M., Bäckman, S., Wikström, P., and Sandstedt, K. (2010). Anthrax outbreak in a Swedish beef cattle herd- 1st case in 27 years: case report. *Acta Vet Scand*, 52: 7.
- Little, SF., and Knudson, GB. (1986). Comparative efficacy of *Bacillus anthracis* live spore vaccine and protective antigen vaccine against anthrax in the guinea pig. *Infect Immun*, 52: 509-512.
- Mongoh, MN., Dyer NW., Stoltenow, CL., and Khaitsa, ML. (2008). Risk factors associated with anthrax outbreak in animals in North Dakota, 2005: A retrospective case-control study. *Public Health Rep*, 123: 352-359.
- Mongoh, MN., Dyer, NW., Stoltenow, CL., Hearne, R., and Khaitsa, ML. (2008). A review of management practices for the control of anthrax in animals: the 2005 anthrax epizootic in North Dakota-case study. *Zoonoses Public Health*, 55: 279-290.
- Nagarajan, K., Selvaraj, J., Balakrishnan, G., Manimaran, K., Padmanath, K., Senthil, N. R. & Parimal, R. (2015) Confirmation of acute nitrate poisoning differentiating from anthrax in three Indian indigenous cattle. *J Adv Vet Anim Res*, 2, 30-33.
- OIE. 2008. OIE listed diseases and other diseases of importance to international trade. Part-2, Chapter 2-1.1. Anthrax; pp. 135-144.
- Roy, P., Rashid, MM., Ferdoush, MJ., Dipti, M., Chowdhury, MGA., Mostofa, MG., Roy, SK., Khan, MAHNA., and Hossain, MM. (2013). Biochemical and immunological characterization of anthrax spore vaccine in goat. *Bangl J Vet Med*, 11: 151-157.
- Sterne, M. 1939. The use of anthrax vaccines prepared from avirulent (uncapsulated) variants of *Bacillus anthracis*. *Onderstepoort J Vet Sci Anim Ind*, 13: 307-312.
- Turnbull, PC., Tindall, BW., Coetzee, JD., Conradie, CM., Bull, RL., Lindeque, PM., and Huebschle OJ. (2004). Vaccine-induced protection against anthrax in cheetah (*Acinonyx jubatus*) and black rhinoceros (*Diceros bicornis*). *Vaccine*, 22: 3340-3347.
- Uddin, MM. (2011). Impact of anthrax on beef price and consumption in Bangladesh: A descriptive analysis. <http://www.ifcndairy.org/extranet/stories.php>. (Accessed on February 2, 2015)
- Weiss, MM., Weiss, PD., and Weiss, JB. (2007). Anthrax vaccine and public health policy. *Am J Public Health*, 97: 1945-1951.
- World Health Organization (web.). Vaccine Immunology, Section-1: General aspects of vaccination. http://www.who.int/immunization/documents/Elsevier_Vaccine_immunology. Accessed February 01, 2015.
- World Health Organization, 2008. Anthrax in Animals and Humans. Available at: http://www.who.int/csr/resources/publications/anthrax_webs.pdf. Accessed November 25, 2011.